

Measles



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Measles

9.0. Measles

9.1. WHO Measles Genotyping Project

Niteen Wairagkar, Sunil Vaidya

Title of Project: Genotyping Of Measles Virus Strains Circulating In Various Parts Of India,

Funding agency : WHO-Initiative on Vaccine Research, Geneva

Objective

➤ Genotype mapping of indigenous measles strains circulating in various parts of India.

Experimental Design

This is being accomplished by investigating representative outbreaks of measles, representative sporadically occurring measles cases from various parts of the country. This is done with the help of collaborators of MeaslesNetIndia. In the NIV laboratory, serology, virus isolations using tissue culture methods and Sequencing N and H genes of different strains of measles is being carried out. Attempts would be made to cover representative areas of the country.

9.1.1. Establishment of MeaslesNetIndia network

In order to accomplish the project task, a voluntary participatory network of 16 centers in India is formed and named as MeaslesNetIndia, at a WHO-India funded workshop on 17 and 18th Oct 2005 in Pune. These centers include ICMR centers, medical colleges and other research centers giving geographical representation in the country. This network has started functioning and we investigated few outbreaks with our study collaborators. MeaslesNetIndia Collaborators:

- National Institute of Virology, Pune
- National Institute of Epidemiology, Chennai
- National Institute of Cholera and Enteric Diseases, Kolkata.
- Regional Medical Research Centre, Dibrugarh
- Rajendra Memorial Research Institute of Medical Sciences, Patna
- Desert Medicine Research Centre, Jodhpur
- Regional Medical Research Centre (ICMR), Andaman & Nicobar Islands,
- Regional Medical Research Centre for Tribals, Jabalpur
- Regional Medical Research Centre, Bhubaneswar
- NIV, Bangalore Field Unit, Bangalore
- Department of Virology, Manipal Medical College, Manipal
- Pasteur Institute, Coonoor
- Dept of Preventive Social Medicine, Government Medical College, Nagpur
- Department of Microbiology, BJMC, Ahmedabad
- Department of Virology, PGI, Chandigarh
- Sanjay Gandhi Post Graduate Institute, Lucknow

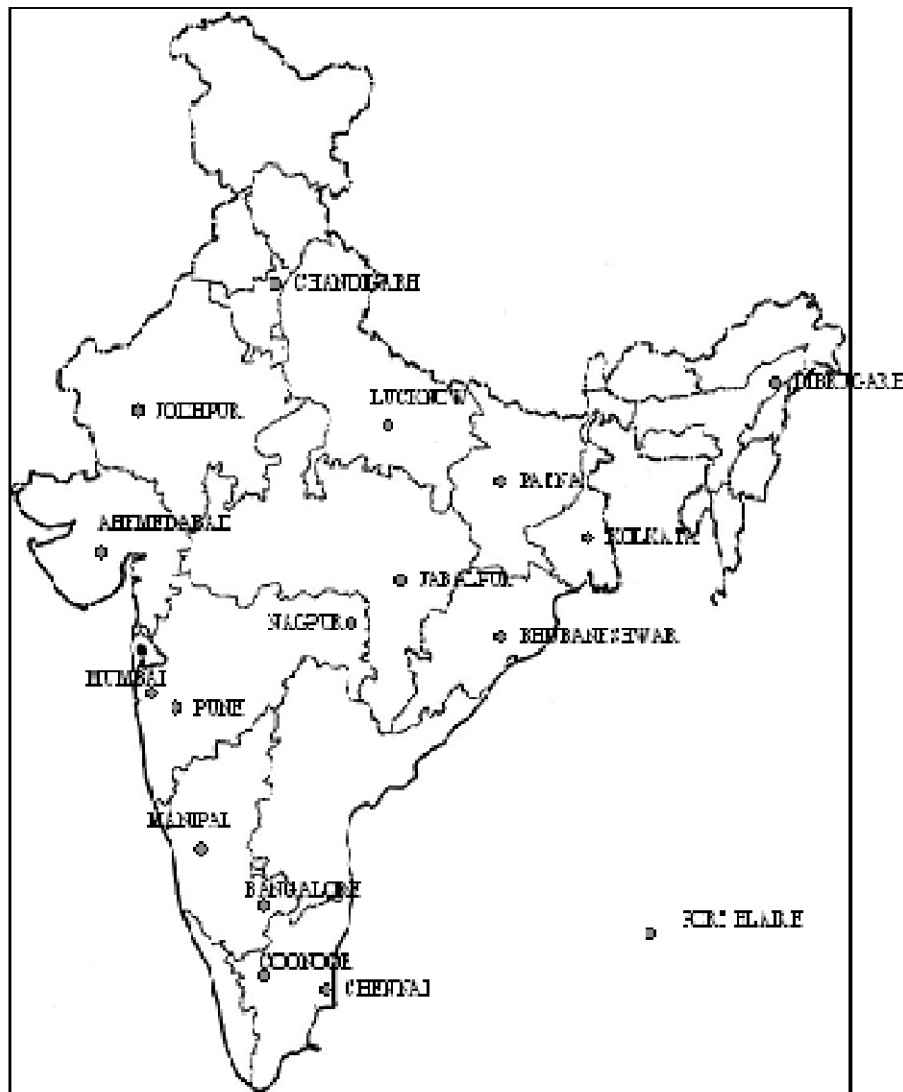


Fig. 1 : MeaslesNetIndia

9.1.2. MEASLES Genotyping studies by MeaslesNetIndia

Genotyping of Measles strains in pre and post-Tsunami disaster in coastal area of Tamil Nadu, India

Sunil Vaidya, Niteen Wairagkar

Manoj Murhekar, National Institute of Epidemiology, Chennai

During Tsunami disaster, thousands of people were displaced causing major population movement in shortest time. An outbreak of measles in the district of Cadlore was detected in post-tsunami phase. 101 cases of measles were detected. Mean age of affected individuals was 5.1 years (range four months to 21 years). The age distribution of cases was as follows: 4% below 9 months; 56.4% between 9 months to 5 years; 38.6 % between 6-15 years and 1% above 15 years. Of the 101 cases, 53 were females (53%). Total 8 Sera and 5 TS

were collected by NIE, Chennai and referred to NIV for further virological work. Out of these 5 sera were IgM positive by ELISA. RT-PCR and sequencing was performed on 2 TS collected from patients. Molecular epidemiological studies on post tsunami outbreak revealed etiological agent as Measles genotype D8. Our earlier studies from Tamil Nadu state show circulation of measles genotypes D4, D7 & D8 in year 2003 and 2004.

Sequencing studies in Satara Outbreak

Sunil Vaidya, Niteen Wairagkar

A small measles outbreak was reported from PHC Masur of Satara district in 2004. Total 12 cases of measles were reported. In current year of reporting, RT-PCR was done on 3 throat swab samples, one Oral Swab, 1 urine, 1 respiratory secretion and 1 Skin swab. Sequencing and phylogenetic analysis detected Measles D4 genotype from three measles cases (TS) and two sequences of D8 genotype from one encephalitis case (RS and OS). This is for the first time we could get sequences directly from the respiratory secretions of an encephalitis case.

Genotypes in Manipal, Karnataka

Sunil vaidya, Niteen Wairagkar

Arunkumar, Manipal Medical College, Manipal.

Measles outbreak was suspected in Manipal in Kasturba Medical college hostel. 12 Serum samples and 4 Throat wash and 3 TCF from 12 adults with fever and rash were referred to NIV. IgM ELISA was positive in 2 cases. 4-throat wash, two serum and 3 TCF were subjected to RT-PCR. One RT-PCR positive sample subjected to partial sequencing, revealed genotype D8-like sequence that is very much diverse from the current circulating measles D8 genotypes. Further studies are in progress.

Genotypes in Bihar

Niteen Wairagkar, Deepika Khedekar, Sarika Raibagkar, Naseem Shaikh

VNR Das, MeaselsNetIndia, Rajendra Memorial Research Institute and Medical Science, Patna.

A total of 5 outbreaks were reported from Bihar. First outbreak had occurred in Pathera village of Supoul district, (near Nepal border-300 km away from RMRIS Patna) affecting 17 patients with 3 deaths. These cases occurred from 12 Dec 23 Dec 2005. Two representative serum samples were collected. Immunization status of the patients was not known. IgM ELISA was performed on these samples. Both were positive for Measles IgM antibodies.

Second outbreak occurred in same district in Shreepur Sardartola village affecting 35 patients with 2 deaths. Two representative serum samples were referred & processed for Measles IgM Elisa. Both were positive. The patients belonged to 3-5yrs age group & were not immunized.

Third outbreak occurred in Chhausa village of Sitamarhi district affecting 30 cases from 29 Dec 2005 to 15 Jan 2006. 2 serum samples 2 throat swabs and 2 urine samples were received. One serum sample was IgM

positive. Virus isolation is in process. RT-PCR of the throat swabs was negative.

Fourth outbreak occurred in Patna district affecting 11 cases. Age group of patients ranged from 2yrs-11yrs. 3-throat swabs, 3 urine sample & 3 serum samples were received. All three were IgM positive. RT-PCR was positive in one throat swab sample. Phylogenetic analysis showed presence of measles D8 genotype. Virus isolation is under process. This patient was immunized for Measles while other two were not immunized.

Fifth outbreak occurred in Mirzapur village of Patna district affecting 120 patients. Immunization history of these patients was not available. 12 serum, 12 urine and 12 throat swabs were referred. 7 serum samples were processed for Measles IgM ELISA in which 6 were positive while one was negative. 12 throat swabs from this second episode were processed for RT-PCR. Four sequences of D8 genotypes were detected in this outbreak.

Genotypes in Bijnor (Uttar Pradesh)

Niteen Wairagkar, Deepika Khedekar, Sarika Raibagkar, Naseem Shaikh

V. Vashishtha, Pediatric specialist, Bijnour.

Suspected measles cases admitted in, Mangala Hospital, Bijnor U.P were included in the study. Ten patients were admitted in the hospital in two time clusters in February, 2006. 10 sera, 10 throat swabs and 7 urine samples of 10 patients were received from this hospital. Age group of these cases ranged from 9 months-11 yrs. All except three patients were un-immunized. Serum samples of these patients were also positive for Measles IgM ELISA. 4 throat swabs and 3 urine samples were processed for RT-PCR. Eight throat swabs were positive for Measles virus. Sequence analysis showed presence of measles genotype D4 (3 cases) and D8 (5 cases). This showed the co-circulation of D4 and D8 genotypes in Bijnor at the same time.

Genotypes in Port Blair, Andaman

Niteen Wairagkar, Deepika Khedekar, Sarika Raibagkar, Naseem Shaikh

A.P.Sugunan, MeaslesNetIndia collaborator- Regional Medical Research Center, Andaman.

In Jan 2006, Brigeganj intermediate shelter had measles transmission detected by pediatricians. Brigeganj relief camp. 3 patients had received vaccine while vaccination status of one patient was not known. 2 serum samples were Measles IgM ELISA positive. Virus isolation and RT PCR is in process. Four sequences of D8 genotype were obtained.

Genotypes in Pune

Niteen Wairagkar, Deepika Khedekar, Sarika Raibagkar, Naseem Shaikh, L.V.Hungund

Measles cases from various hospitals in and around Pune were investigated by the Measles Group. Throat swabs of 11 patients were processed for RT-PCR, of which 6 were positive for measles virus. 5 corresponding serum samples tested positive for IgM ELISA and while one was negative. Serum samples of remaining 5 patients were positive for Measles IgM ELISA. Sequences of these 6 RT-PCR positive samples showed presence of measles genotypes D4 (1case), D8 (4 cases), and D7 (1 case). Out of available four urine samples, 2 samples tested positive for RT-PCR. Virus isolation was attempted from three throat swabs. Virus isolates obtained were also processed for RT-PCR. All three were positive. The sequences were similar to their corresponding clinical sample sequences (Fig. 2).

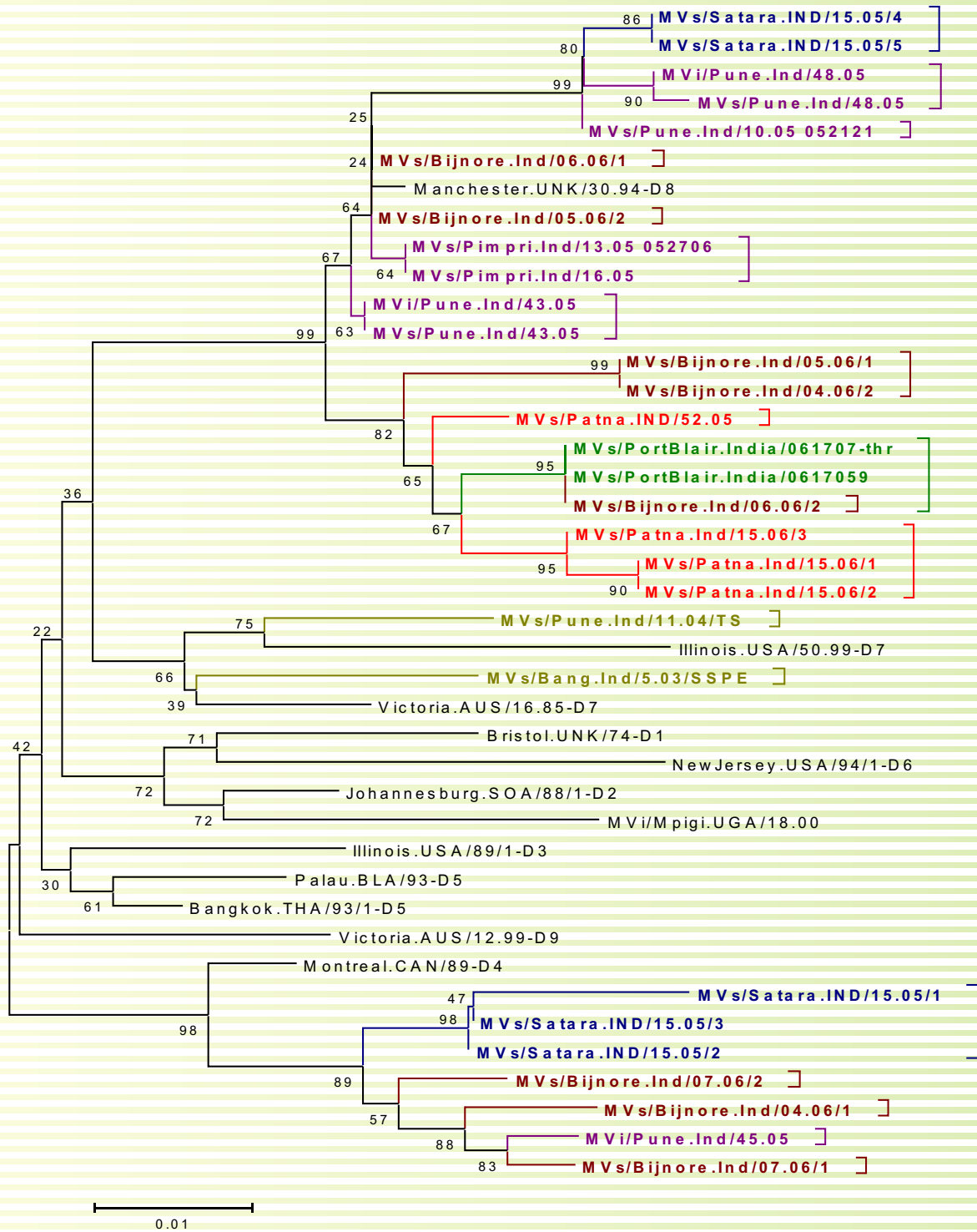


Fig. 2 : Phylogenetic tree of Indian Measles strains (WHO genotyping Project).

Genotyping from SSPE case from Bangalore

Sunil Vaidya, Niteen Wairagkar

S.Shankar, National Institute of Mental Health and Neuro Sciences, (NIMHANS) Bangalore.

An 18-year-old adult male from a North Karnataka State of South India presented with weakness of right side of the body for 2 weeks, altered sensorium for one week and seizures for 3 days. After various studies at NIMHANS Bangalore, clinical samples were referred to NIV with suspicion of Fulminant SSPE. Serum sample was positive by IgM ELISA. Autopsied brain tissue sample was subjected to RT-PCR and sequencing. Phylogenetic analysis revealed the presence of measles genotype D7 (Fig. 3). This suspected SSPE measles N gene sequence is 94% identical to the WHO reference D7 genotype (Illinois) where as 96% identical with recently genotyped Pune sporadic D7 genotype. Sequence obtained in this study found 94% identical with earlier studied D7 sequence from Chennai, south India.

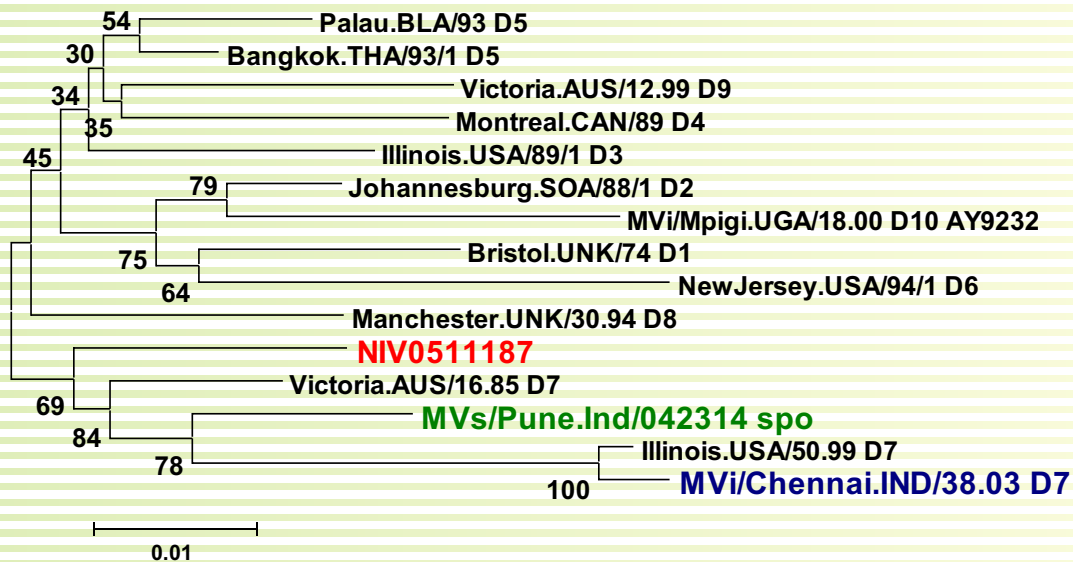


Fig. 3 : Genotype D based phylogenetic tree of Measles N gene

9.1.3. Establishment of Indian Measles Sequence Database

This is being done at the Measles Group at NIV with development of integrated database management Software. This database will give every information about the sequence, viz from the clinicoepidemiological, geographical to the results of all the tests. This database will be extensively used and would be extremely useful for Tracking transmission pathways of Measles strains in India. At present this database contains over 50 sequences from Indian measles strains.

9.2 WHO Measles Aerosol Vaccine Trial

Niteen Wairagkar, Naseem Shaikh

KEM and NARI, Pune

NIV was selected as the Principal site for WHO-ICMR initiated Phase 1 vaccine trial entitled, Safety of an aerosol attenuated Measles Vaccine (Open, non-controlled, sequential trial, by age group), for the period of two years (December 2005 to October 2007). One of the aerosol device, selected by WHO, would be tried in Pune. Total 60 subjects (20 each from 18-35 years, 5-17 years and 1-4 years old) would be given EZ measles vaccine manufactured by Serum Institute of India and would be followed for one year with 12 visits for safety assessment.

Extensive work has gone into preparation for this trial project, preparation of documents like, Measles Aerosol Vaccine Protocol, Investigator's Brochure, Informed consent forms, Patient Information Sheets, SOPs for various procedures etc. The Generic protocol converted into site specific protocol, submitted to various committees like local ethics committees of three collaborators, DCGI, WHO ERC, ICMR, HMSC for approvals. The Good Clinical Practices (GCP) trainings and Ethics trainings were conducted. Pre-trial clinical monitor's visit and WHO evaluation of the site has been done. Actual recruitment of the subjects is likely to start in April, 2006.

NIV Measles Lab is evaluated and recognized as Central Measles Reference Lab for all three sites for this project. NIV Measles group standardized PRNT as basic test for this project. The lab also participated in International efforts along with 6 global labs for standardization of SOP for PRNT for use in measles vaccine trial. This is for the first time such International collaboration was sought for vaccine trial. NIV scientific and technical staff were trained at Health Protection Agency, Colindale, UK, on PRNT for this vaccine trial. Subsequently, NIV lab was also evaluated by HPA scientist. QA panel was successfully evaluated by the lab. The Clinical biochemistry and HIV screening protocols were standardized with NARI.

9.3 WHO-NIBSC Collaborative study for Preparation of WHO III anti measles International Sera.

Niteen Wairagkar, Naseem Shaikh

NIV Measles group was requested to collaborate in this study in view of the expertise in PRNT for measles. This study was initiated to characterize the candidate sera pool for preparation and standardization as Measles International sera. 12 Global laboratories from 8 countries (4 National Control Labs, 4 Vaccine manufacturers, 4 Public health Labs), including NIV measles lab, were invited to carry out Measles assays on the blind coded sera panel supplied by NIBSC. The results from these 12 labs were analyzed to find out suitability of the Candidate pool as WHO III anti measles International serum. NIV results were well received and were within the ranges as compared to other global labs.

Measles